## REMARKS

Further and favorable reconsideration is respectfully requested in view of the foregoing amendments and following remarks.

By this Amendment, claims 1-36 are cancelled and new claims 37-42 are added.

Entry of the amendments is proper under 37 CFR §1.116 because the amendments: (a) place the application in condition for allowance (for the reasons discussed herein); (b) do not raise any new issue requiring further search and/or consideration (as the amendments address issues previously discussed throughout prosecution); (c) do not present any additional claims without cancelling a corresponding number of finally rejected claims; and (d) place the application in better form for appeal, should an appeal be necessary. Entry of the amendments is thus respectfully requested.

New claim 37 corresponds to present claim 18. However, the definition of $R_1$, $R_2$, $R_3$ and $R_4$ of the dihydrotriazine compound of the formula (la) in new claim 37 is restricted to specific substituent(s), as shown below:

$R_1$ is (i) a phenyl group, (ii) a benzyl or 2-phenylethyl group which is optionally substituted by methyl or methoxy, (iii) a quinolyl group or (iv) a cyclohexylmethyl group;

$R_{21}$ is n-octyl, n-nonyl or n-decyl; and

$R_3$ and $R_4$ are each methyl.

In new claim 39, the term "gluconate" of 4-octylamino-3,6-dihydro-6,6-dimethyl-2-(4'-methylbenzylamino)-1,3,5-triazine has been deleted, because the term "gluconate" corresponds with the term "a salt thereof."

Support for new claim 40 can be found in the compound of Example 65 of the specification.

The relationship between the new claims and the present claims (and the example from the specification) is shown below.

| Amended claims | Present claims and *(Example No.)* |
|---|---|
| 37 | 18, 21, *(1,2,4,37,38(2),48,49 56,58,65,82 and 83*)* |
| 38 | 22, 23, *(1,2,4,37,38(2),48,49 56,58 and 83*)* |
| 39 | 25 *(58)* |
| 40 | (newly added) *(65)* |
| 41 | 34 |
| 44 | 35 |

*The compound of Example No. 83 is 4-octylamino-3,6-dihydro-6,6-dimethyl-2-phenylamino-l,3,5-triazine acetate [Compound No. (12) described in Declaration (2) Under 37 CFR 1.132, which is discussed below].

## The Characteristics of the Present Invention

Claim 37 recites the combination of:

(A) an amino group mono-substituted by (i) a phenyl group, (ii) a benzyl or 2-phenylethyl group which is optionally substituted by methyl or methoxy, (iii) a quinolyl group or (iv) a cyclohexylmethyl group **at the 2-position**; and

(B) an amino group mono-substituted by n-octyl, n-nonyl or n-decyl **at the 4-position** in the dihydro-1,3,5-triazine compound of formula (la).

Based on the structural characteristics, the dihydrotriazine compounds of formula (la) of the present invention exert surprising and unexpected effects of sterilizing or disinfecting, and are useful as external bactericides and disinfectants.

In accordance with the guidance of FDA regulations (i.e., FDA Register: June 17, 1994, Part III Department of Health and Human Services, Food and Drug Administration, Sec. 333.470 Testing for Health-care Antiseptic Drug Products) (enclosed), the present inventors conducted tests of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of many of the dihydro-l,3,5-triazine compounds of the present invention. The MBC test is conducted by contacting the microorganism with the test compound for a short time (e.g., 0.5, 1, 3, 5 minutes).

The FDA guidance mentions assay methods of drugs for use as antiseptic handwash or health-care personnel handwash, patient preoperative skin preparation and surgical hand scrub.

Namely, (l) MIC test, (2) determining the emergence of resistance of microorganisms to drugs, and (3) time-kill studies have to be conducted. An assessment of how rapidly the antimicrobial product produces its effect is required for an external bactericidal/disinfectant agent. When an external bactericidal/disinfectant agent is actually used, the contact time of drugs with microorganisms is 1 to 30 minutes, and therefore the time-kill studies are to include enumerations at times 0, 3, 6, 9, 12, 15, 20 and 30 minutes.

The MIC tests of diaminodihydrotriazine derivatives have been reported so far, but there have not been any MBC tests of dihydro-1,3,5-triazine compounds reported. This is the first time that the present inventors have included this information in the present application.

**The Submission of Two Declarations Under 37 CFR 1.132**

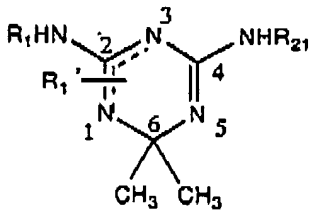
To show the surprising and unexpected effects of sterilizing or disinfecting of the dihydrotriazine compounds of formula (la) of claim 37, Applicants submit two Declarations under 37 CFR 1.132 (Declaration (1) and Declaration (2)) duly executed by Dr. Shirou Maeda, the first named inventor of the present application.

Declaration (1)

In Declaration (1), bactericidal activities of compound Nos. (1) - (11) were compared with that of the known bactericide, chlorhexidine (a 20% chlorhexidine digluconate solution was used). The results are shown in Tables 1-7 of Declaration (1). These experiments are also disclosed in the working Examples of the present specification.

Table I (below) describes test compounds of the present invention. For comparison, Table II (below) shows the MBC values (μg/ml) against (1) *S. aureus* 209PJC, (2) MRSA 97-115, (3) *E. coli* NIHJ JC-2 and (4) *P. aeruginosa* PAO-1 of test compound Nos. (1) - (11) and chlorhexidine.

For comparison, Table III (below) shows the MBC values against (5) MRSA KM 97-53, (6) MRSA KM97-108, (7) VRE 49, (8) P. *aeruginosa* No. 12 and (9) *P. aeruginosa* KM97-5 of test compound Nos. 6 and 9, and chlorhexidine.



$R_1'$ : H

Table I

| Compd. No. | $R_1$ | $R_{21}$ | salt | Ex. No. |
|---|---|---|---|---|
| (1) | 4-methoxybenzyl | decyl | HCl | 1 |
| (2) | benzyl | decyl | $CH_3SO_3H$ | 2 |
| (3) | 4-methoxyphenethyl | decyl | HCl | 4 |
| (4) | 4-methybenzyl | octyl | (free-form) | 37 |
| (5) | 4-methoxybenzyl | decyl | $CH_3SO_3H$ | 38(2) |
| (6) | benzyl | nonyl | $CH_3CO_2H$ | 48 |
| (7) | benzyl | nonyl | HBr | 49 |
| (8) | phenethyl | nonyl | $CH_3CO_2H$ | 56 |
| (9) | 4-methybenzyl | octyl | $CH_3CO_2H$ | 58 |
| (10) | cyclohexylmethyl | octyl | $CH_3CO_2H$ | 65 |
| (11) | 4-quinolyl | octyl | $CH_3CO_2H$ | 82 |

Table II

| | Minimal Bactericidal Concentration (MBC) ($\mu$g/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Compound Nos.(1)-(11) | | | Chlorhexidine | | |
| | 1 minute | 3 minutes | 5 minutes | 1 minute | 3 minutes | 5 minutes |
| ① | 6.3 - 50 | 3.1 - 12.5 | 3.1 - 6.3 | 62.5 | 62.5 | 62.5 |
| ② | 12.5 - 50 | 6.3 - 25 | 6.3 - 25 | 1000 | 250 | 125 |
| ③ | 3.1 - 12.5 | 1.6 - 6.3 | 1.6 - 6.3 | 62.5 | 31.3 | 15.6 |
| ④ | 1.6 - 12.5 | 0.8 - 12.5 | 0.8 - 6.3 | >400 | >400 | >400 |

(1) *S. aureus* 209JPC

(2) MRSA 97-115

(3) *E. coli* NIHJ JC-2

(4) *P. aeruginosa* PAO-1

Table III

| | Minimal Bactericidal Concentration (MBC) (μg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Compound Nos.(6) and (9) | | | Chlorhexidine | | |
| | 1minute | 3minutes | 5minutes | 1minute | 3minutes | 5minutes |
| ⑤ | 50 | 25 - 50 | 25 | 500 | 250 | 250 |
| ⑥ | 50 | 50 | 25 | 500 | 500 | 500 |
| ⑦ | 25 | 12.5 - 25 | 12.5 | >1000 | >1000 | >1000 |
| ⑧ | 6.3 - 25 | 6.3 - 12.5 | 3.1-12.5 | 125 | 32 | 32 |
| ⑨ | 3.1 - 6.3 | 3.1 | 3.1 | 31 | 16 | 8 |

(5) MRSA KM 97-53

(6) MRSA KM97-108

(7) VRE 49

(8) P. *aeruginosa* No. 12

(9) P. *aeruginosa* KM97-5

It is quite clear from Tables II and III that the MBC values of compound Nos. (1) - (11) of the present invention are much smaller than those of chlorhexidine.

Accordingly, compound Nos. (1) - (11) of the present invention show much stronger bactericidal activity than chlorhexidine. These results are surprising and unexpected to one of ordinary skill in the art.

Declaration (2)

In Experiment 3 of Declration (2), bactericidal activity at a contact time of 0.5 minutes of the compounds of the present invention was compared with that of several known compounds and structurally close dihydro-l,3,5-triazine compounds, which do not fall within the scope of claim 37.

The contact time of 0.5 minutes, which is clearly shorter than 1 minute, was adopted to evaluate the prompt efficiency of the test compounds more clearly.

Among the present compounds, Compound Nos. (9) and (10), and 4-octyamino-3,6-dihydro-6,6-dimethyl-2-phenylamino-l,3,5-triazine acetate (Compound No. 12) were chosen as the representatives. Compound No. (12) falls within the scope of the claims as originally filed, but was not specifically described in the specification. Compound No. (12) was newly prepared

as the representative of 1,3,5-triazine having a phenylamino substituent at the 2-position in accordance with the disclosure in the specification.

As the comparative compounds, compounds (A) to (K) were selected, as follows:

Compound (A): Trimethoprim

Compound (B): Chlorhexidine digluconate

Compound (C):
2,4-Diamino-3,6-dihydro-6,6-dimethyl-1-(4'-chlorophenyl) 1,3,5-triazine acetate [Compound 1 of Journal Medicinal Chemistry 1977, vol.20(No.2) 237-243]

Compound (D):
2,4-Diamino-3,6-dihydro-6-n-pentadecyl-1,3,5-triazine hydrochloride

Compound (E):
4-Amino-3,6-dihydro-6,6-dimethyl-2-benzylamino-1,3,5-triazine acetate

Compound (F):
4-Amino-3,6-dihydro-6-methyl-2-dimethylamino-1,3,5-triazine hydrochloride

Compound (G):
2,4-Di-(n-propylamino)-3,6-dihydro-6,6-dimethyl-1,3,5-triazine hydrochloride

Compound (H):
2-(4-t-Buthylphenylamino)-4-n-buthylamino-3,6-dihydro-6,6-dimethyl-1,3,5-triazine acetate

Compound (I)
2,4-Di-(dimethylamino)-3,6-dihydro-6-n-decyl-6-methyl-1,3,5-triazine hydrochloride

Compound (J)
2,4-Di-(dimethylamino)-3,6-dihydro-6-undecyl-6-dimethyl-1,3,5-triazine hydrochloride and

Compound (K)
4-(Octyl(methyl)amino)-3,6-dihydro-6,6-dimethyl-2-(benzyl(methyl)amino)-1,3,5-triazine hydrochloride

The comparative compounds can be classified into the following five groups:

i) 1,3,5-triazine having an amino substituent at the 2- and 4-positions:
Compounds (C) and (D)

ii) 1,3,5-triazine having a mono-substituted amino substituent at the 2-position and an amino substituent at the 4-position:
Compound (E)

iii) 1,3,5-triazine having a di-substituted amino substituent at the 2-position and an amino substituent at the 4-position:
Compound (F)

iv) 1,3,5-triazine having a mono-substituted amino substituent at the 2- and 4-positions:
Compounds (G) and (H)

v) 1,3,5-triazine having a di-substituted amino substituent at the 2- and 4-positions:
Compounds (I), (J) and (K)

Please note that Compounds (D) - (K) are not specifically described in Moinet, but Moinet's disclosure broadly covers Compounds (D) - (K).

The results are shown in Table 2 of Declaration (2).

For comparison, Table IV (below) describes the MBC values (μg/ml) of test Compound Nos. (9), (10) and (12), and Compounds (B) - (D) and (F) - (K) [please also see Table 2 in Declaration (2)].

**Table IV**

| | Minimal Bactericidal Concentration (MBC) (μg/ml) | |
|---|---|---|
| | Compound Nos.(9),(10) and (12) Contact time: 0.5 min. | Compounds (B)-(D), (F)-(K) Contact time: 0.5 min. |
| ① | 25 to 50 | >100 to >200 |
| ② | 25 to 50 | >100 to >200 |
| ③ | 12.5 to 25 | 25 to >200 |
| ④ | 25 | 25 to >200 |

(1) *S. aureus* 209JPC

(2) MRSA 97-115

(3) *E. coli* NIHJ JC-2

(4) *P. aeruginosa* PAO-1

**It is quite clear from Table IV that MBC values of Compound Nos. (9), (10) and (12) of the present invention are much smaller than those of comparative Compounds (B) - (D) and (F) - (K).**

Further, Compound (A) shows >50 to >100 of MBC at a contact time of 5 minutes [see Table 2 of Declaration (2)]. Therefore, the MBC at a contact time of 0.5 minutes is expected to be greater than that at a contact time of 5 minutes.

**Accordingly, Compound Nos. (9), (10) and (12) of the present invention show much stronger bactericidal activity than known bactericides (A) - (B) and structurally similar 1,3,5-triazine compounds (C), (D) and (F) - (K). These results are surprising and unexpected to one of ordinary skill in the art.**

Further, in Experiment 2 of Declaration (2), the antibacterial activity of the compounds of the present invention was compared with several known compounds and structurally close dihydro-1,3,5-triazine compounds. Table 1 of Declaration (2) demonstrates that Compounds (A), (B), (G) - (K) and Compound Nos. (9), (10) and (12) show strong antibacterial activity, compared with compounds (C), (D), (E) and (F).

It is clear that the antibacterial activity shown by MIC value does not always coincide with the bactericidal activity shown by MBC value. These results are surprising and unexpected to one of ordinary skill in the art.

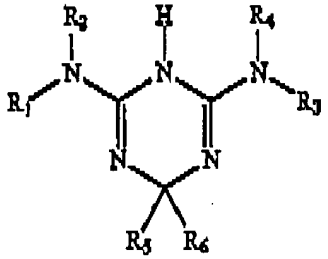
## Claim Rejections Under 35 U.S.C. § 103

The Examiner rejects claims 18, 21-24, 27, 28, 30, 34 and 35 under 35 U.S.C. § 103(a) as being unpatentable over Moinet (WO 01/55122; U.S. 2003/0109530, hereinafter "Moiniet '530"), and again rejects claims 18, 21-24, 27, 28, 30, 34, and 35 under 35 U.S.C. § 103(a) as being unpatentable over Moinet '530; and rejects claims 18, 21-24, 27, 28, 30, 34 and 35 under 35 U.S.C. § 103(a) as being unpatentable over Moinet (U.S. 7,034,021, hereinafter "Moiniet '021"). Claims 18, 21-24, 27, 28, 30, 34 and 35 are cancelled, rendering the rejection moot. However, as applied to new claims 37-42, Applicants respectfully traverse the rejections.

To find a *prima facie* case of obviousness, a known compound may suggest its homologue, analogue, or isomer because such compounds often have similar properties, and therefore a chemist of ordinary skill in the art would ordinarily contemplate making them to try to obtain compounds with improved properties. However, in order to find a *prima facie* case of unpatentability **in such instances, a showing that the prior art would have suggested making the specific molecular modifications necessary to achieve the claimed invention is also required**. Moreover, in cases involving new chemical compounds, **it is necessary to identify some reason that would have led a chemist to modify a known compound in a particular manner to establish *prima facie* obviousness of a new claimed compound.** See *Takeda Chemical Industries v. Alphapharm*, 492 F.3d 1350, 1356-1357 (Fed. Cir. 2007) (copy enclosed).

Moinet provides no reason or rationale for one of ordinary skill in the art to have made the specific molecular modifications to Moinet's compounds to achieve the compounds of claim 37 of the present invention.

Moinet discloses the compounds of the formula (I) **that are useful in the treatment of pathological conditions associated with insulin-resistance syndrome**:



wherein $R_1$, $R_2$, $R_3$ and $R_4$ are chosen independently from the groups:
(1) H, (2) (C1-C20)alkyl, (3) (C2-C20)alkylene, (4) (C2-C20)alkyne, (5) (C3-C8)cycloalkyl, (6) (C3-C8)heterocycloalkyl, (7) (C6-C14)aryl(C1-C20)alkyl, (8) (C6-C14)aryl, and (9) (C1-C13)heteroaryl, or (10) it being possible for $R_1$ and $R_2$, to form with the nitrogen atom an n-membered ring (n between 3 and 8), or (11) it being possible for $R_3$ and $R_4$ to form with the nitrogen atom an n-membered ring (n between 3 and 8), wherein each substituent is optionally substituted with specified groups (see columns 1-2 of Moiniet '021, and page 1, paragraphs [0006]-[0016] of Moiniet '530).

Accordingly, each of $R_1$, $R_2$, $R_3$, and $R_4$ can be substituents (1) - (11). Therefore, a great number of combinations of substituents $R_1$, $R_2$, $R_3$, and $R_4$ are present in the compounds of formula (I) of Moinet.

Moinet broadly and vaguely covers the combination of a group of "$R_1HN$-" at the 2-position and a group of "-$NHR_{21}$" at the 4-position of the dihydro-1,3,5-triazine compounds of the present invention.

However, Moinet does not specifically disclose the specific combination of "$R_1HN$-" at the 2-position and "-$NHR_{21}$" at the 4-position of the dihydrotriazine compounds, as recited in claim 37 of the present invention. Furthermore, Moinet does not teach or suggest compounds of formula (I) as antibacterial agents for sterilizing/disinfecting (claims 41 and 42).

With regard to the disclosure of specific combinations of $R_1$ to $R_4$ of Moinet, please see the disclosures on column 3, lines 53-56 of Moiniet '021, and page 2, paragraph [0041], lines 1-4 of Moiniet '530, which states, "A more particular group of compounds of formula (I) is that in which R1 and R2 are chosen independently from the groups specified above with the exception of the hydrogen atom and R3 and R4 represent a hydrogen atom." Also see column 3, lines 57-59 of Moinet '021, and page 2, paragraph [0041], lines 4-7 of Moinet '530, which states, **"a preferred group of compounds of formula (I) is that in which R1 and R2 are an alkyl, advantageously methyl group and R3 and R4 represent a hydrogen"** (emphasis added). **Accordingly, Moinet teaches that an amino group at the 4-position is preferred.**

Further, in the Examples in Table II on columns 7-22 of Moinet '021, and on pages 4-12 of Moinet '530, the compounds having the following combination of substituents at the 2- and 4-positions are specifically disclosed.

| | The 2-position | The 4-position | Compound No. |
|---|---|---|---|
| (1) | dimethylamino | amino | 1, 3-7, 10-13, 18 19, 24, 25, 27-45 |
| (2) | dimethylamino | dimethylamino | 2, 15 |
| (3) | dimethylamino | 1-propen-3-ylamino | 8, 26 |
| (4) | dimethylamino | isopropylamino | 9 |
| (5) | dimethylamino | ethylamino | 14, 23 |
| (6) | dimethylamino | methylamino | 16, 22 |
| (7) | dimethylamino | pyrrolidin-1-yl | 17, 21 |
| (8) | phenylethylamino (i.e. phenethylamino) | amino | 20 |

Moinet specifically teaches only the combinations of (1) - (8), as shown in the above table for the treatment of pathological conditions associated with insulin-resistance syndrome.

**Therefore, Moinet does not teach or suggest the specific combination recited in claim 37 of:**

(a) an amino group mono-substituted by (i) a phenyl group, (ii) a benzyl or 2-phenylethyl group which is optionally substituted by methyl or methoxy, (iii) a quinolyl group or (iv) a cyclohexylmethyl group of $R_1$ at the 2-position; and

(b) an amino group mono-substituted by n-octyl, n-nonyl or n-decyl of $R_{21}$ at the 4-position.

Accordingly, Moinet does not provide any reason or rationale for one of ordinary skill in the art to make the specific molecular modifications necessary to achieve the claimed invention. Therefore, a *prima facie* case of obviousness has not been established.

The Unexpected Effects of the Present Invention

The compounds of the present invention have surprisingly unexpected effects in terms of sterilizing or disinfecting (please see Declarations (1) and (2), as discussed above, and page 32, lines 19-28 of the specification).

On the other hand, Moinet '021 mentions on column 4, lines 40-42 (and page 3, paragraph [0048] in Moinet '530) that "The compounds according to the present invention are useful in the treatment of pathological conditions associated with the insulin-resistance syndrome (X syndrome)." Further, in column 4, lines 57-64 of Moinet '021 (and Moinet '530 mentions on page 3, paragraph [0052]) the reference teaches that "The compounds according to the present

invention can also be used to treat chronic complication which are in particular due to the formation of 'advanced glycosylation end-products' noted AGEs which are derived from the glycoxidation reaction between glucose, its oxidation derivatives and the amino functional groups of proteins, including the so-called Maillard reactions for glycation of glyoxal for example."

Thus, the effects of sterilizing or disinfecting of the present invention are quite different from that of treatment of pathologies related to the insulin-resistance syndrome and treatment of chronic complication of Moinet, and thus are surprising and unexpected.

Furthermore, it is clear from Declaration (1) that Compound Nos. (1) to (11) of the present invention show much stronger bactericidal activity than chlorhexidine, and it is clear from Declaration (2) that Compound Nos. (9), (10) and (12) of the present invention show much stronger bactericidal activity than known bactericides (A) - (B) and structurally close dihydrotriazine compounds (C), (D) and (F) - (K).

These are surprising and unexpected effects that would not have been obvious to a person skilled in the art.

Therefore, the presently claimed invention would not have been obvious over the Moinet references.

## Conclusion

In view of the foregoing, it is respectfully submitted that this application is in condition for allowance. Favorable reconsideration and prompt allowance of the claims are earnestly solicited.

Should the Examiner find that anything further would be desirable in order to place the application in better condition for allowance, the Examiner is invited to contact the undersigned at the telephone number set forth below.

Respectfully submitted,

Shirou MAEDA et al.

By: 

Andrew B. Freistein
Registration No. 52,917
Attorney for Applicants

Enclosures: (1) FDA Register: June 17, 1994, Part III Department of Health and Human Services, Food and Drug Administration, Sec. 333.470 Testing for Health-Care Antiseptic Drug Products
(2) Declaration (1) Under 37 CFR 1.132
(3) Declaration (2) Under 37 CFR 1.132
(4) *Takeda Chemical Industries v. Alphapharm*, 492 F.3d 1350 (Fed. Cir. 2007)

WMC/ABF/rgf
Washington, D.C. 20005-1503
Telephone (202) 721-8200
Facsimile (202) 721-8250
June 15, 2009

[Federal Register: June 17, 1994]

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**Part** III

Department of Health and Human Services

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Food and Drug Administration

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**21 CFR** Parts **333** and 369

Tentative Final Monograph for Health-Care Antiseptic Drug Products; Proposed Rule
DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

**21 CFR** Parts **333** and 369

[Docket No. 75N-183H]
RIN 0905-AA06

# Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Tentative Final Monograph for Health-Care Antiseptic Drug Products

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice of proposed rulemaking.

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SUMMARY: The Food and Drug Administration (FDA) is issuing a notice of proposed rulemaking in the form of an amended tentative final monograph that would establish conditions under which over-the-counter (OTC) topical health-care antiseptic drug products are generally recognized as safe and effective and not misbranded. FDA is issuing this notice of proposed rulemaking to amend the previous notice of proposed rulemaking on topical antimicrobial drug products (see the Federal Register of January 6, 1978, 43 FR 1210) after considering the public comments on that notice and other information in the administrative record for this rulemaking. FDA is also requesting data and information concerning the safety and effectiveness of topical antimicrobials for use as hand sanitizers or dips. This proposal is **part** of the ongoing review of OTC

drug products conducted by FDA.

**Sec. 333.470 Testing of health-care antiseptic drug products.**

**(a) General testing criteria.** The procedures in this section are designed to characterize the effectiveness of antiseptic drug products formulated for use as an antiseptic handwash or health-care personnel handwash, patient preoperative skin preparation, and surgical hand scrub. Requests for any modifications of the testing procedures in this section or alternative assay methods are to be submitted in accordance with paragraph (d) of this section.

**(1) In vitro testing.** The following tests must be performed using the antiseptic ingredient, the vehicle, and the finished product for all drug product classes:

**(i)** Determine the in vitro antimicrobial spectrum of the active ingredient, the vehicle, and the final formulation using both standard cultures and recently isolated strains of each species. A series of recently isolated mesophilic strains, including members of the normal flora and cutaneous pathogens (50 isolates of each species, half of which must be fresh clinical isolates), are to be selected.

**(ii)** Determine the **minimal inhibitory concentrations (MIC)** using methodology established by the National Committee for Clinical Laboratory Standards and entitled ``Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically,'' Document M7-A2, 2d ed., 10:8, 1990, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 **CFR part** 51. Copies are available from the National Committee for Clinical Laboratory Standards, 771 East Lancaster Ave., Villanova, PA 19085, or may be examined at the Center for Drug Evaluation and Research, 7520 Standish Pl., suite 201, Rockville, MD, or the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC. Twenty-five fresh clinical isolates and 25 laboratory strains of the organisms listed in this section are to be included. All in vitro tests must include the American Type Culture Collection (ATCC) reference strains (available from American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852) specified in paragraphs (a)(1)(ii)(A) and (a)(1)(ii)(B) of this section. The agency requires that these organisms be used in testing unless data can be presented to the agency that other organisms are equally representative of organisms associated with nosocomial infection. There must be no claims, either direct or by implication, that a product has any activity against an organism or that it reduces the number of organisms for which it has not been tested. The following organisms are to be included (note: special media and environmental conditions may be required):

(A) Gram negative organisms: Acinetobacter species; Bacteroides fragilis; Haemophilus influenza; Enterobacter species; Escherichia coli (ATCC Nos. 11229 and 25922); Klebsiella species, including Klebsiella pneumonia; Pseudomonas aeruginosa (ATCC Nos. 15442 and 27853); Proteus mirabilis; and Serratia marcescens (ATCC No. 14756).

(B) Gram positive organisms: Staphylococci: Staphylococcus aureus

(ATCC Nos. 6538 and 29213); Coagulase-negative Staphylococci: Staphylococcus epidermidis (ATCC No. 12228), Staphylococcus hominis, Staphylococcus haemolyticus, and Staphylococcus saprophyticus; Micrococcus luteus (ATCC No. 7468); and Streptococci: Streptococcus pyogenes, Enterococcus faecalis (ATCC No. 29212), Enterococcus faecium, and Streptococcus pneumoniae.

(C) Yeast: Candida species and Candida albicans.

**(iii)** Determine the possible development of resistance to the chemical. Two approaches to **determining the emergence of resistance** to a particular antimicrobial are to be used. The first approach involves a determination of the evolution of a point mutation by the sequential passage of an organism through increasing concentrations of the antimicrobial included in the culture medium. The second approach is a thorough survey of the published literature to determine whether resistance has been reported for the antimicrobial ingredient. The survey is to include information on the microbial contamination of marketed products containing the antimicrobial ingredient in question irrespective of drug concentration. The survey is to cover all countries in which products containing the active ingredient are marketed. Any information submitted in a foreign language should include a translation. Alternate approaches to determining the development of resistance can be submitted as a petition in accord with
Sec. 10.30 of this chapter. The petition is to contain sufficient data to show that the alternate approach provides a reliable indication of the development of resistance to a particular antimicrobial ingredient.

**(iv) Time-kill studies.** (A) The assessment of the in vitro spectrum
of the antimicrobial provides information on the types of genera and species that may be considered susceptible under the conditions of the test procedure described in paragraph (a)(1)(ii) of this section. **However, information is also required that allows an assessment of how rapidly the antimicrobial product produces its effect.** Such information
may be derived from in vitro time-kill curve studies using a selected battery of organisms and a specified drug concentration.

(B) The satisfactory performance of the test product as assessed by
the results of the MIC studies, the time-kill studies, and the simulated in vivo clinical trials of organisms representing the resident microbial flora can then be used to assess the effectiveness of the test product for the transient microbial flora most commonly encountered in the clinical setting. This procedure is required because
methods, other than the health-care personnel hand test, do not exist for assessing the in vivo effectiveness of test products versus the transient microbial flora.

(C) It is recognized that a generally accepted or standardized method that may be used in conducting in vitro time-kill studies is not
available, but **the agency encourages the submission of proposed methods
that may be considered applicable to this test.** Many variables that should be considered in the development of a method have been addressed
for antibiotics and are also applicable to these products. Such variables are described by Schoenknecht, F. D., L. D. Sabath, and C. Thornsberry, ``Susceptibility Tests: Special Tests,'' in the ``Manual

of Clinical Microbiology,'' 4th ed., edited by E. H. Lennette et al., American Society for Microbiology, Washington, pp. 1,000-1,008, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 **CFR part** 51. Copies are available from the American Society for Microbiology, Washington, DC, or may be examined at the Center for Drug
Evaluation and Research, 7520 Standish Pl., suite 201, Rockville, MD, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(D) The procedure to be used is to incorporate the recommendations described on page 1,004 of the chapter in the ``Manual of Clinical Microbiology'' cited in paragraph (a)(1)(iv)(C) of this section with the following modifications. Because the time frames of greatest interest for antiseptic drug products intended for health-care personnel handwash, surgical hand scrub, and patient preoperative skin preparation use are **1 to 30 minutes,** the time-kill studies are to focus
on these time frames and are to **include enumerations at times 0, 3, 6, 9, 12, 15, 20, and 30 minutes.** Enumerate the bacteria in the sampling solution by a standard plate count procedure such as that described in ``Standard Methods for the Evaluation of Dairy Products'' (available from American Public Health Association, Inc., 1015 15th St. NW., Washington, DC 20005), but using soybean-casein digest agar and a suitable inactivator for the antimicrobial where necessary. The suitability of the inactivator is to be demonstrated using a procedure such as described in E 1054, ``Test Methods for Evaluating Inactivators
of Antimicrobial Agents Used in Disinfectant, Sanitizer, and Antiseptic
Products,'' in ``Annual Book of ASTM Standards,'' vol. 11.04, which is incorporated by reference in accordance with 5 U.S.C. 552(a) and 1 **CFR part** 51. Copies are available from The American Society for Testing and
Materials, 1916 Race St., Philadelphia, PA 19103-1187, or may be examined at the Center for Drug Evaluation and Research (HFD-810), 5600
Fishers Lane, Rockville, MD, or at the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC. The battery of organisms selected is to represent the resident microbial flora most commonly encountered under actual use conditions of the test product and the transient microbial flora most likely to be encountered by health-care professionals in clinical settings. Therefore, the micro-organisms to be used in these time-kill studies are to be the standard ATCC strains identified in paragraph (a)(1)(ii) of this section. The drug concentration to be tested should be a tenfold dilution of the finished product.

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Michael R. Taylor,
Deputy Commissioner for Policy.
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